AD NUMBER

AD400115

NEW LIMITATION CHANGE

TO

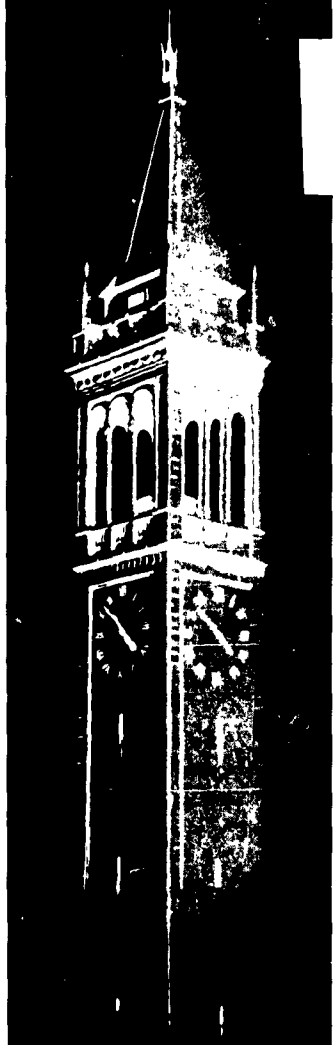
Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 31 OCT 1962. Other requests shall be referred to Air Force Rome Air Development Center, Rome, NY.

AUTHORITY

radc ltr, 15 jun 1967



RADC-TDR-62-624

Nonthermal Effects of Microwave Radiation

by
C. Süsskind and Staff

Series No. 60, Issue No. 489
Contract Nos. AF41(657)-114 and Nonr-222(92)
October 31, 1962

ELECTRONICS RESEARCH LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

"This is to certify that the experiments were conducted according to the 'Principles of Laboratory Animal Care' of the National Society of Medical Research."

"When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely related Government procurement operation, the United States Government thereby incurs no responsibility nor any obligation whatsoever and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto."

"Qualified requesters may obtain copies of this report from the ASTIA Document Service Center, Arlington Hall Station, Arlington 12, Virginia. ASTIA Services for the Department of Defense contractors are available through the 'Field of Interest Register' on a need-to-know certified by the cognizant military agency of their project or contract."

RADC-TDR-62-624

Electronics Research Laboratory
University of California
Berkeley, California

NONTHERMAL EFFECTS OF MICROWAVE RADIATION

Annual Scientific Report (1961-62) on Contract No. Nonr-222(92)
and Final Report (1957-62) on Contract No. AF 41(657)-114

by

C. Süsskind and Staff

Institute of Engineering Research
Series No. 60, Issue No. 489

The research reported in this document
was sponsored jointly by
Rome Air Development Center under Contract No. AF 41(657)-114
and Office of Naval Research under Contract No. Nonr-222(92)

October 31, 1962

SUMMARY

Chronic irradiations of mice with 3-cm microwaves, carried out at the University of California during the past several years, have shown no clear effects on longevity at the sublethal doses employed. Some other effects, notably with regard to cancer of the white cells and testicular damage, resulted from this pilot study. A detailed description of the results appears in Trans. IRE BME-9: 104-108, 1962.

This report also contains a summary of the results obtained by P. O. Vogelhut in irradiating enzymes at the same wavelength; these results are described in greater detail in another report (Issue No. 476) in this Series.

TABLE OF CONTENTS

LIST OF FIGURES

SCOPE OF THE REPORT

The present report is the final report under Contract AF 41(657)-114 and the first annual report under Contract Nonr 222(92). The contract sponsored by the U. S. Air Force on cellular and longevity effects of microwave radiation was carried on at this Laboratory from 1 December 1956 to 15 March 1962. The U. S. Navy contract on the nonthermal effects of microwave radiation began on 1 September 1961. Because of the overlaps in the objectives, the periods, and the personnel of the two projects during 1961-62, both sponsoring agencies have agreed that the reports covering that period should be consolidated.

Detailed descriptions of the work performed under U. S. Air Force sponsorship in previous years are contained in four Annual Scientific Reports issued in Series 60 of the University of California's Institute of Engineering Research, as follows:

1957-58: "Biological effects of microwave radiation," Issue No. 205, 30 June 1958.

1958-59: "Cellular and longevity effects of microwave radiation," Issue No. 241, 30 June 1959.

1959-60: "Microwave radiation as a biological hazard and tool," Issue No. 285, 30 June 1960.

1960-61: "Longevity study of the effects of 3 cm microwave radiation on mice," Issue No. 382, 30 June 1961.

In addition, the project was represented at several of the Tri-Service Conferences on microwave hazards, as follows:

1958 (Rome, N. Y.): "Effects of microwave irradiation on internal temperature and viability in mice."

1959 (Berkeley, Calif.): "Physical aspects of microwave radiation"; "Temperature regulation in laboratory animals irradiated with 3-cm microwaves"; and "Analytical and experimental investigation of unicellular organisms with 3-cm microwaves."

1960 (New York, N. Y.): "Longevity and cellular studies with microwaves."

1961 (New York, N. Y.): "Effects of chronic microwave irradiation on mice."

The last paper was presented at the 1961 International Conference on Medical Electronics (where several sessions had been set aside to take place of the Tri-Service Conference) and subsequently appeared in the Institute of Radio Engineers Transactions on Bio-Medical Electronics (BME-9: 104-108, 1962), a reprint of which is included as Appendix I in the present report.

I. EXPERIMENTS WITH LABORATORY ANIMALS (S. Prausnitz)

A. Longevity Study

The experiment to determine pathological and longevity effects caused by chronic microwave irradiation of mice was initiated several years ago. Calibration procedures were developed during the first year and involved mainly determining the LD_{50} levels at various irradiation rates. The decision to carry out the longevity test at one half the LD_{50} was made quite early, but many combinations of radiation power density and duration of exposure combine to yield an LD_{50}. The combination finally chosen was selected on the basis of the capabilities of the available transmitter and the length of irradiation suitable for repeated exposures (0.100 w/cm^2, 4.5 min). A total of 300 mice participated in the experiment--200 irradiated and 100 controls--but the final conclusions were based on a somewhat smaller number, since some of the mice that died (either as a result of the irradiation or from natural causes) had undergone post mortem changes that were too extensive to permit histological assessment.

The principal results were that irradiation with 3.2-cm microwaves at half the LD_{50}, 5 days per week for 59 weeks, had no discernible effect on the longevity, weight, or blood picture of the mice; that about 3 times as many irradiated mice had cancer of the white cells as controls; and that about 4 times as many irradiated mice exhibited testicular damage as controls.

A detailed description of the experimental results appears in Appendix I.

B. Liver Enzyme Inactivation by Microwaves

A pilot study of the effect of microwave energy on enzyme activity in mouse livers was carried out to determine whether microwaves might be used as the source of heat in such an experiment, and whether any inactivating effect over and above that caused by heat could be ascribed to microwaves. Although the effects of temperature on enzyme activity have been studied for over a century,[1,2] none of the studies utilized microwave power as the source of heat.

Two major effects operate[1-3] when an enzyme is exposed to increased temperatures: (1) an accelerating effect upon the catalytic action of the enzyme,

and (2) inactivation of the enzyme owing to denaturation of the enzyme protein. The first effect predominates below the optimum temperature (i. e., the temperature at which maximum activity occurs) and the second above it. Fenton and Cowgill[4] point out that most enzymes are destroyed above 80°C and that a point of balance between acceleration of catalytic effect and destruction of the enzyme usually lies between 40 and 50°C.

The source of all liver used in the experiment was a colony of adult male Swiss Albino mice (NAMRU strain) weighing between 30 and 40 g. The animals received a standard pellet diet and were given tap water to drink, both of which were administered ad libitum. The source of microwave power was a U. S. Air Force AN/TPS-10D radar transmitter operating in 1-μsec pulses at a wavelength of 3.2 cm (9270 Mc). Liver temperatures were taken by means of a small glass-covered Veco thermistor, which formed one arm of a dc unbalanced bridge, the output of which was connected to a Varian Model G-10 graphic recorder.

The experimental procedure was as follows: the animals were sacrificed between 9:30 and 10:30 a. m. by a sharp blow on the head, whereupon one lobe of the liver was immediately removed and placed inside a polystyrene holder to be irradiated (Fig. 1). One liver at a time was exposed to a given power density; power densities employed ranged between 0.100 and 0.615 w/cm^2. All livers were exposed for 15 min. Temperature readings were not taken during irradiation because the presence of the thermistor in the microwave field was found to alter the normal temperature gradient within the liver. Instead, the thermistor probe was inserted within 3-5 sec after the power had been turned off, and was left in to describe a cooling curve from which the maximum temperature reached previously could be extrapolated. After exposure the liver tissue was weighed on a torsion balance and placed in a covered Petri dish with 100-percent humidity, to be incubated overnight at a temperature of 34-36°C. This pilot study was limited to enzyme inactivation (though it is recognized that a more complete investigation would have to take the rate of enzyme kinetics into account) and therefore the livers were incubated overnight so that all enzyme activity could reach completion. The lobes of wet liver used weighed between 364 and 787 mg. Control livers underwent identical handling, except that the power was not turned on.

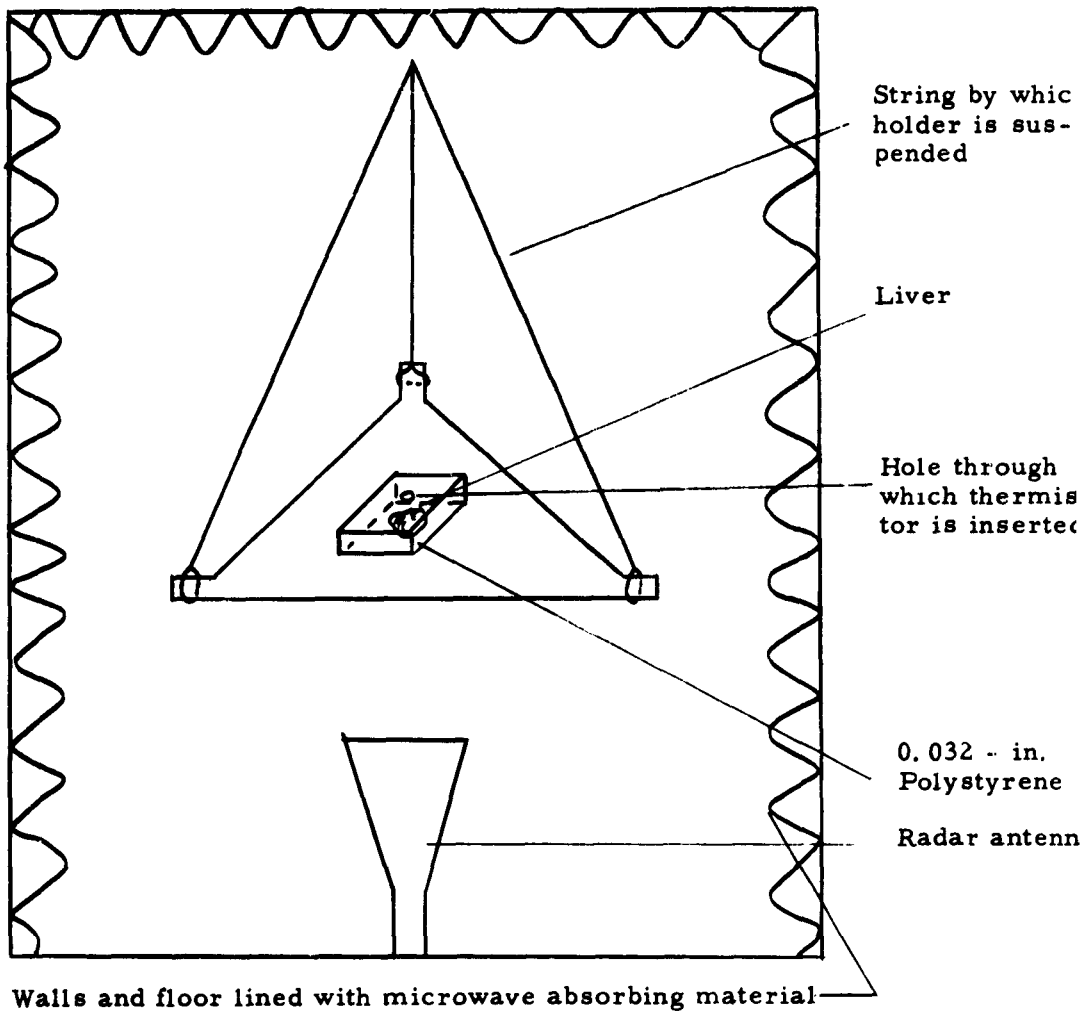


FIG. 1-- Microwave irradiation set-up.

The next morning each liver was placed in 2 ml of a 30-percent solution of KOH, heated, and then precipitated with alcohol and dissolved in water for glycogen determination by the anthrone method modified from Green and Wade.[5] The method is based on formation of a characteristic color of glucose with anthrone in concentrated sulfuric acid which is read in a Klett-Summerson colorimeter. The standard used was an aqueous solution of glucose of known concentration. The results for liver glycogen in the present report do not contain a correction factor for blood glucose.

Six treated and 2 control livers were usually analyzed simultaneously. In all, 6 power densities, and consequently 6 temperature ranges, were studied. In addition to the controls receiving handling identical to that of the irradiated livers, 2 control groups were analyzed in an effort to determine the maximum concentration of glycogen possible. One was a group of 5 livers which were dropped into the KOH solution within 30 sec of death; the other was a group of 5 livers which underwent a 30-min wait between removal and analysis, to represent the time delay that occurred before livers to be irradiated could be placed into the experimental set-up.

The results show that no apparent enzyme inactivation took place when the tissue temperature was raised as high as 49°C, whereas elevation to 85°C or higher resulted in complete inactivation in the average liver. Between 49° and 85°C progressive inactivation took place as the maximum temperature reached by the liver increased (Fig. 2). No incubated controls showed glycogen when analyzed; controls tested within 30 sec and after 30 min yielded a mean value of 5.26 and 5.01 g glycogen/100 g wet tissue, respectively. It was concluded on the basis of the control results that at room temperature a delay as long as 30 min between the time of death of the animal and analysis of the tissue for glycogen brings about essentially no diminution in the amount of glycogen present. The figure 5.26 g glycogen/100 g wet tissue was used to represent 100 per cent glycogen in the liver.

The conclusions of this pilot study are as follows. Inactivation by microwave energy of the liver enzymes that are concerned with glycogen breakdown appears to be brought about in the same manner as has been described for ordinary heat inactivation. The enzymes were found to be completely destroyed when exposed to a power density high enough to bring about a maximum tissue temperature of approximately 85°C in 15 min; glycogen

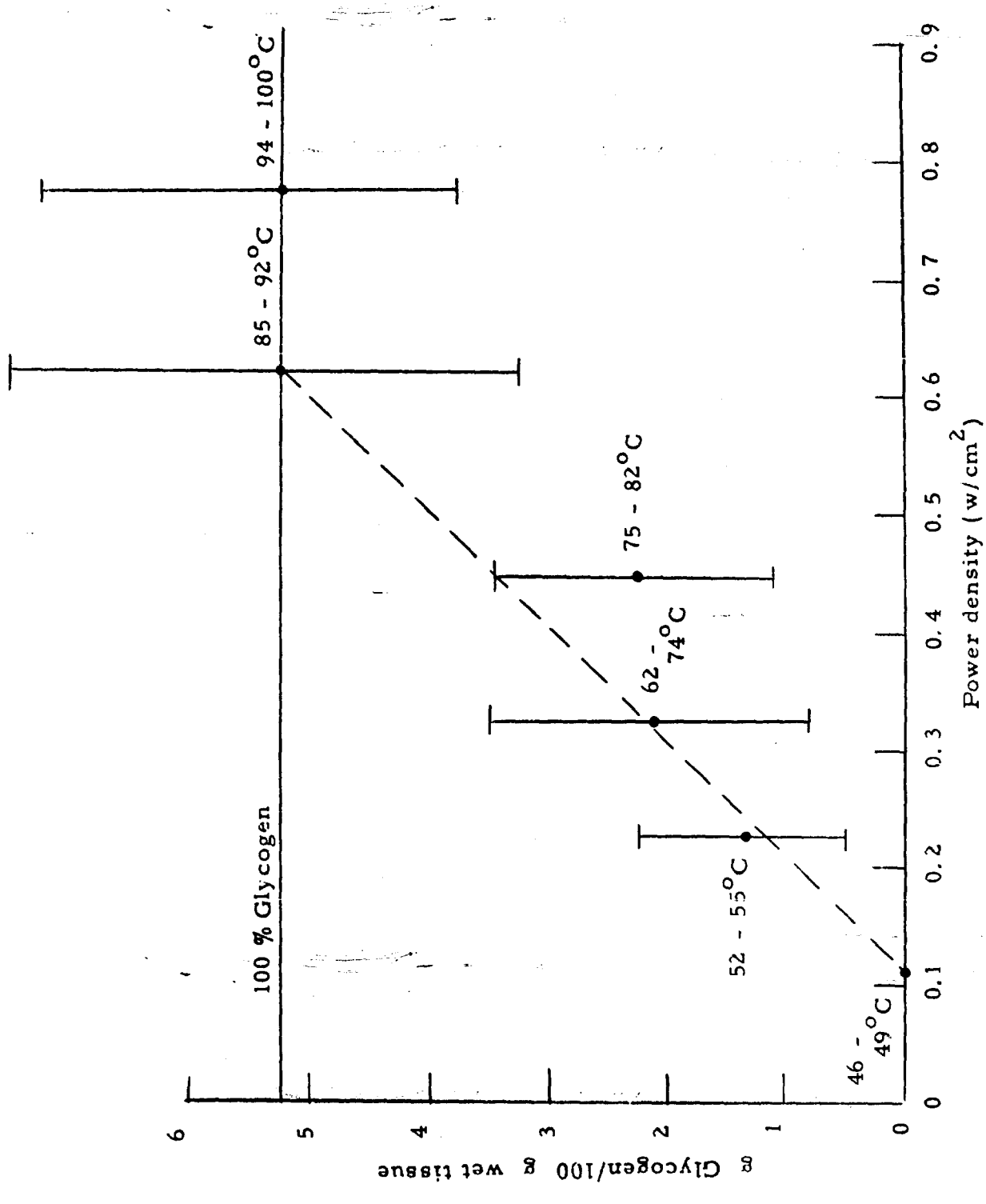


FIG. 2-- Amount of glycogen remaining in livers irradiated with microwave energy and subsequently incubated overnight. The numbers next to each point (about 5 livers for each point) represent the maximum temperatures reached during irradiation. The vertical lines show the standard deviation.

breakdown was complete at temperatures below 49°C. These figures substantiate those in the literature obtained by purely thermal methods.[4]

It is important to note that complete glycogen digestion could be brought about by a small percentage of enzyme left after an irradiation that only partially destroyed the enzyme. Overnight incubation is sufficient for glycogen breakdown by reduced enzyme activity to occur. A further study that would concern itself with enzyme kinetics would show whether any enzyme inactivation has indeed occurred at temperatures below 49°C; it would also reveal any accelerating effects upon the catalytic action of the enzyme. Between 49° and 85°C the amount of glucose left undigested is probably a function of the fraction of liver which becomes so thoroughly cooked that no enzyme activity at all is left. Here again, a study of enzyme kinetics would detect whether any enzyme inactivation has occurred below the cooking temperature.

The relatively large standard deviations shown in Fig. 2 can be explained by the variability of maximum temperatures reached at each power density point. A part of this variability may be the result of extrapolating to the maximum temperature reached, but the primary cause would seem to be variability in tissue mass and heat capacity.

The aid of Duane Blume and Dr. Paola Timiras in guiding this investigation and their many helpful suggestions are gratefully acknowledged.

II. EXPERIMENTS WITH ENZYMES (P. O. Vogelhut)

The study of the effect of microwaves on enzymes, like the experiments with laboratory animals, had its beginnings in the investigation of possible hazards to personnel operating high-power microwave equipment. The investigation explored several possibilities of the effects of electromagnetic waves on living specimens. A mere listing of such effects was deemed to be too superficial; rather, research was directed towards the explanation of the phenomena observed, or listed in the literature. The analysis of all the recorded effects strongly suggested that they could be explained by the occurrence of a fundamental interaction between the constituents of living matter and electromagnetic waves. The basic constituent of living organisms that was further investigated was protein and water under the influence of electromagnetic radiation. In particular, the real and imaginary parts of the

dielectric constants were measured, since here was an effect of electromagnetic radiation that was unequivocally in existence and constituted the most basic form of interaction that could be conveniently described. This approach proved to be most fruitful and produced accurate data concerning the electrical behavior of enzyme molecules while they are interacting with their substrate molecules; the result was a general description of the electrical and chemical reactions on the surfaces of macromolecules. Many effects of electromagnetic radiation that could not be previously explained could now be subjected to a more critical examination in the light of the hypothesis of a fundamental interaction between living matter and such radiation.

A review by Jaski and Susskind,[6] made as part of the present investigation in 1961, indicated that nonthermal effects could actually exist in a variety of systems and different levels of organization of biological matter. The present investigation attempted to formulate a theory that could explain all these various effects by demonstrating the existence of an influence of electromagnetic radiation capable of affecting all these systems to a major or minor extent. One fact is common to all biological systems: they consist of aqueous solutions of protein molecules. These molecules are the most significant ones because they can act as enzymes and can therefore control the metabolism of the single cell or organism of which they are the building blocks. The scope of the problem was therefore reduced to the investigation of the effect of electromagnetic waves on aqueous solutions of enzymes. To find this effect one only had to note what, if any, property of these solutions could be measured by means of radio waves, since any measurement implies an interaction between the instrument and the thing to be measured. One property of protein solutions that can quite easily be measured with electromagnetic radiation is the dielectric constant.

The above reasoning can be illustrated with reference to Fig. 3. Experiments performed by several investigators at the two commonly employed frequencies of 70 and 3000 Mc are plotted. The axis labelled "S" indicates the complexity of the system, which to a first approximation is considered to be proportional to the volume that the system occupies. The other axis, D, indicates the dose that has to be delivered to the system to produce a certain minimum effect. As can be seen from the graph, both sets of experiments exhibit a linear relationship. This behavior is interpreted to mean

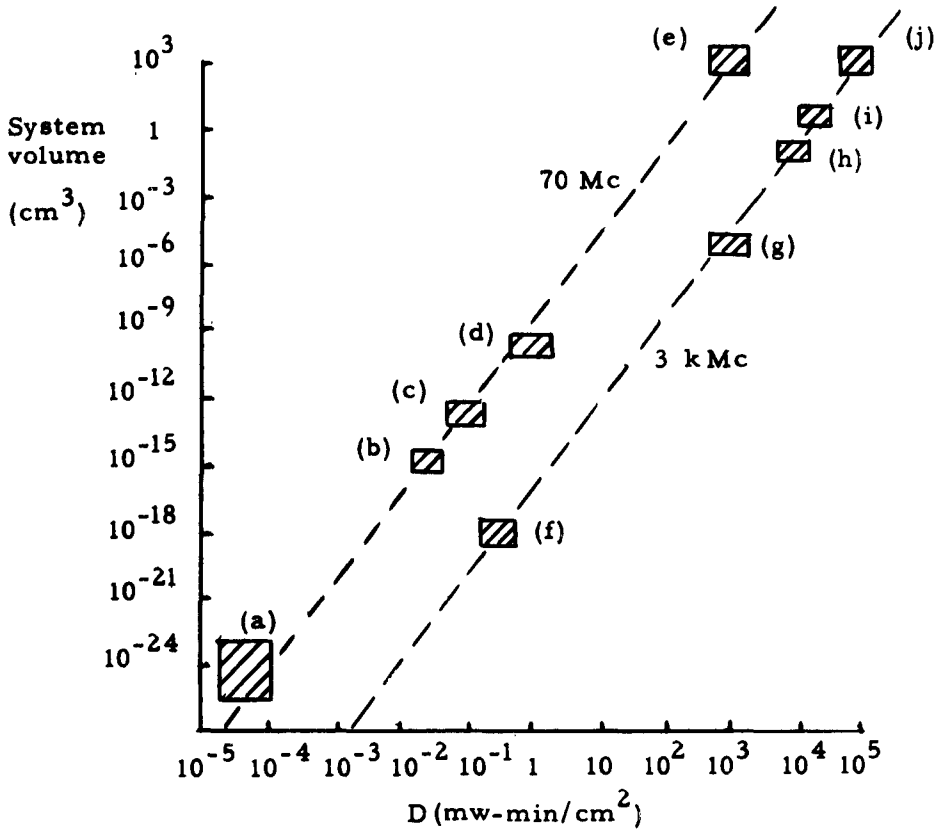


FIG. 3-- Approximate relationship between size of irradiated material and rf dosage necessary to produce an effect. (a) Fumarase (from A. de Pereira Forjaz, Biochem. Z. 280: 53, 1936); (b) serum proteins (from W. W. Lepeschkin, Biochem. Z. 318: 15, 1945); (c) single E coli (from H. Fleming, Elec. Eng. 63: 18, 1944); (d) colonies of E coli (ibid.); (e) guinea pigs and dogs (from C. H. Addington et al., Proc. Fourth Tri-Service Conf. Biol. Effects of Microwave Radiation, Plenum Press, New York, 1961; p. 177); (f) glycogen (from W. A. G. van Everdingen, Ned. Tijdschr. Geneesk. 82: 1873, 1938; 84: 4370, 1940; 85: 3094, 1941; and 87: 406, 1943); (g) fruit fly larvae (from G. W. Searle et al., Proc. Fourth Tri-Service Conf. Biol. Effects of Microwave Radiation, Plenum Press, New York, 1961; p. 187); (h) mice (from van Everdingen, op. cit.); (i) rats (from G. W. Searle et al., op. cit.); and (j) dogs (ibid.).

that a basic mechanism exists in each set that can be influenced to some degree by irradiation with electromagnetic waves, and that this influence becomes progressively smaller as the complexity of the system under investigation increases. In other words, as this basic mechanism becomes more and more "diluted" more dosage has to be applied to the system to procure some observable effect of the radiation.

The above relations suggest that it may be the simplest molecular association that has biological significance on all levels of complexity. We suggest that this simplest association of molecules is a protein-water system. This belief is strengthened when we observe that the above-mentioned linear relationship occurs at two widely separated frequencies that also happen to be two regions of the spectrum in which water shows a slight dispersion, as shown in a separate report (see below).[7]

The investigation then proceeded as follows. First, an instrument was developed that could measure the dielectric constants of aqueous solutions of proteins rapidly and accurately in the microwave region of the electromagnetic spectrum. The accuracy and reliability of the measurements were tested by comparison with the data obtained by other investigators. An independent test was established by deducing values of hydration of the proteir molecules from the data obtained in the present investigation and comparing those values with theoretical and experimental estimates.

In view of the apparent correlation of the effectiveness of certain wavelengths in producing biological effects and the observed slight dispersions of the dielectric constant of water in those regions, the dielectric constant of water was then investigated over the complete radio-frequency spectrum and over a range of temperatures; this study yielded a new theory of the dielectric behavior of water.

Next, impurities were introduced into the water system and the resulting changes in the behavior of the dielectric constant of the solution were observed. Protein in solution was treated as a special type of impurity and the theoretical basis for the above-mentioned estimates of hydration of proteins was derived from the dielectric data.

One special group of proteins, namely enzymes, was then considered, with special emphasis on one particular enzyme, pepsin. A new hypothesis of enzyme action was advanced that could explain the changes in dielectric

constants which occurred as the enzyme reacted with its substrate molecule.

The study concluded with the suggestion that radio waves affect protein systems in general, and enzyme systems in particular, in varying degrees according to wavelength.

The detailed investigation is described in a separate report by P. O. Vogelhut, which also formed his doctoral dissertation.[7]

A report on the microwave measurements aspects of the investigation was presented at the Conference on Microwave Measurement Techniques held by the Institution of Electrical Engineers in London on 6-9 September 1961. A reprint of the paper constitutes Appendix II of the present report.

APPENDIX I: EFFECTS OF CHRONIC MICROWAVE IRRADIATION ON MICE

APPENDIX II: CAVITY PERTURBATION MEASUREMENT OF THE EFFECTS OF MICROWAVE RADIATION ON PROTEINS

CAVITY PERTURBATION MEASUREMENT OF THE EFFECTS OF MICROWAVE RADIATION ON PROTEINS

By Prof. C. SÜSSKIND, Ph.D., and P. O. VOGELHUT, B.Sc.

The extensive programme carried out during the past few years in the United States under government (notably U.S. Air Force) sponsorship to determine the biological effects of microwave radiation has been directed primarily at the establishment of criteria that would make such radiation less hazardous to personnel. The programme has also paid dividends in two additional fields. The first is in bio-electronics, which is concerned with the employment of electromagnetic radiation as a tool in the biological sciences; a survey of this growing field was recently published by Jaski and Süsskind.* The second is in microwave measurement techniques, since special methods are often necessary to deal with the unusual conditions dictated by the nature of the sample on which measurements are to be made. The experiment described in this contribution is an example of some of the fascinating problems that arise in this field.

The most obvious effect of microwaves on biological substances is dielectric heating. In the case of mammals, the heating is complicated by the frequency-dependent nature of the penetration of microwave power through the layers of skin, fatty tissue and muscle, and by the fact that most sensors of temperature change lie near the surface, so that deep heating might remain unperceived for a dangerously long time. Measurable body-temperature changes occur when the incident radiation has a power density of the order of $0{\cdot}1\ W/cm^2$. The generally recommended safety level has been set at one-tenth of this value, or $0{\cdot}01\ W/cm^2$, and much of the recent government-sponsored research work in America has been directed at establishing what factors (such as effective radar cross-section, reflectivity owing to clothing, pulsed versus continuous-wave, and on-off versus steady operation and ambient conditions) might force a revision of this safety level. But, regardless of these relatively minor factors, the fact remains that heating is the principal effect observed to date, and it overshadows any other effects of a non-thermal nature which might also be present.

The reason why these non-thermal effects might be important is that such effects as have been observed appear to be much more subtle than the heating; they tend to take place even at levels well below the $0{\cdot}01\ W/cm^2$ 'safe' level. On the other hand, they may not have any biological significance. The problem is threefold: the effect must be observed; it must be precisely measured and accounted for theoretically; and its biological significance must be determined.

Any biological system contains mainly macromolecules and water. Until now it has been very hard to obtain information about the water in these systems and what new properties might arise from the association of these two prime constituents of living matter. The best-known system to date is a protein, which is why proteins were chosen for the present investigation and why the role of water in that system is being investigated. Other systems of enzymes and substrates may have been chosen, but it seemed logical to pick the one about which most was known. At the same time, it is certainly hoped that the tool being developed might be applicable to problems on all three levels of biology: in molecular biology (enzyme-substrate systems), in cellular biology (i.e. in the cybernetic approach to the functioning of the living cell) and in organismic biology (where typical problems might be the transmission of an impulse along a nerve or the processes associated with anaesthesia).

The effects under investigation in the present experiment are changes in the hydration of proteins (and thus of enzymatic reactions and of the specificity of enzymes) as a result of microwave irradiation at a very low power. This quantity is calculated from measurements that yield instantaneous values of the real and imaginary parts of the dielectric constant (and of their time rate of change as the reaction proceeds). The experimental conditions require that relatively high values of the real and imaginary parts (of the order of 60 and 30, respectively) must be obtained within milliseconds and with an accuracy better than 1%, and that the properties of the sample must remain unaffected by the measurement procedure. The technique is an adaptation of the 2-cavity method proposed by Birnbaum and Franeau.*

The instrument is calibrated by measuring the shift (i) in Q-factor, and (ii) in resonant frequency produced, when a liquid with accurately known properties (such as benzene or N-butyl alcohol) is introduced in an empty micropipette placed parallel to the E field (but not at the position of maximum E) in a rectangular cavity operating in the TE_{104} mode. Next, the biological sample is sent through at a constant rate and the measurements are repeated.

From the change in Q-factor and resonant frequency we compute the changes in the real and imaginary parts of the dielectric constants. Other important parameters can then be determined from these quantities, such as the relation between the two parts of the dielectric constant of a particular substance dissolved in water when the concentration of the substance is known.

A parameter which adequately expresses this relationship is the static dielectric constant. It is obtained by completing the semicircles shown in Fig. 1 so that they intersect the $\epsilon' C$ plane. The rate of change of this static dielectric constant with concentration is then interpreted to yield the effective loss of water molecules from the total amount that can rotate *with* the electric field. This loss is mainly associated with the stronger binding forces that exist around the solute molecules. In this way, one arrives at a quantitative estimate of the number of irrotationally bound molecules.

The electronic equipment that makes the measurement of change in resonant frequency and cavity Q-factor possible is shown in Fig. 2. The backward-wave oscillator produces a frequency-modulated signal that is proportional to a sawtooth wave, which is also used to synchronize the oscilloscope. Part of the signal is coupled out and mixed with a local oscillator to produce two i.f. signals, which are detected by a tunable receiver. One signal is the sum of the local-oscillator and signal-generator

* *Science*, 1961, 133, p. 443.

This work was supported by the Air Research and Development Command, U.S.A.F., under Contract No. AF41(657)-114 with the Rome Air Development Center, N.Y.

Prof. Süsskind and Mr. Vogelhut are at the Electronics Research Laboratory, University of California, Berkeley, U.S.A.

* *Journal of Applied Physics*, 1949, 20, p. 817.

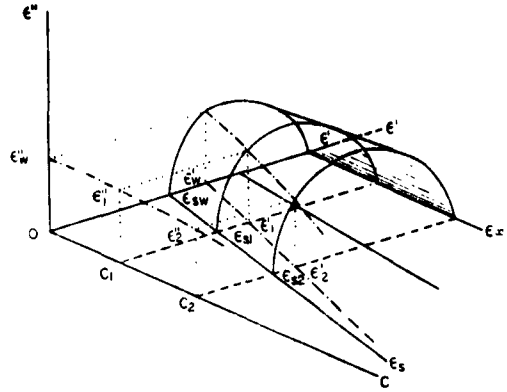


Fig. 1.—Relation between concentration, C, and dielectric constant for a particular substance in aqueous solution.

The method is similar to that of COLE, K. S., and COLE, R. H.: *Journal of Chemical Physics*, 1941, 9, p. 341.

ϵ' Real part of dielectric constant.
ϵ'' Imaginary part of dielectric constant.
ϵ_s Static dielectric constant.
ϵ_∞ Optical dielectric constant.
w Subscript denoting water, i.e. zero concentration.
C Concentration.

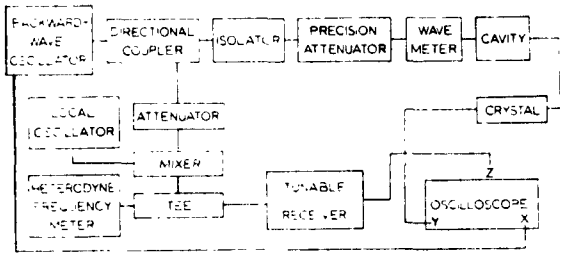


Fig. 2.—Schematic of measurement arrangement.

frequencies, and the other is the difference. The receiver can be calibrated with a heterodyne frequency meter. The i.f. signals are amplified, detected, and used for z-axis (intensity) modulation of the oscilloscope trace to produce intensity markers with accurately known frequency separation in units of frequency. Then the oscilloscope trace is photographed and the response curve of the cavity is superimposed on the same picture.

A rectangular cavity operating in the TE_{104} mode is used (Fig. 3). It is found that changes in Q-factor and resonant

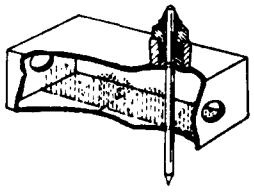


Fig. 3.—Cutaway drawing of TE_{104} cavity, indicating position of sample tube and distribution of electric-field lines.

frequency can be measured with much greater sensitivity if the sample is placed off the position of maximum electric field—in fact, quite near the node. Changes in the electric field vector show up best there; if the sample tube were at the maximum electric field, the resulting attenuation would be too great to yield any signal at all. In order to reduce the attenuation, either the volume or the position of the sample has to be changed.

Fig. 4.—Oscillograms of cavity resonance curves for pure water and for two concentrations of a calibrating solution (benzene).

Shift in resonant frequency f from empty-cavity value (9·5 Gc/s) yields ϵ'. Height y of resonance curve and width of its base give Q-factor and hence ϵ''; calibration markers are obtained by means of precision attenuator and wavemeter (Fig. 2), respectively.

Since the volume cannot be reduced below a practical limit, the position is adjusted instead.

The reason why the TE_{104} mode is used is similarly a practical one: namely to optimize the ratio of cavity volume to sample-tube volume in order to obtain an easily detectable signal. This ratio influences the Q-factor of the system. The better the Q-factor the greater is the signal/noise ratio. An increase in cavity volume increases the Q-factor, whereas an increase in sample volume lowers it; values of these parameters were chosen which optimize the conditions to yield the best signal/noise ratio. The noise itself is due to residual line-frequency modulation (in amplitude and frequency) of the microwave generator and local oscillator. Crystal noise and vibrations also contribute. Similar considerations apply in determining the size of the coupling holes. All these parameters prove to be quite critical. As for the frequency, the only restriction is that it should fall within the range of the local oscillator, which in the present experiment happens to be 9·5 Gc/s.

As has been shown, microwaves lend themselves, after a little coaxing, to investigations of some important problems that exist in biology today. The knowledge of bound water and its behaviour around proteins can be successfully analysed; in addition, such a technique may also prove to be useful in other fields where dielectric constants at these frequencies are to be measured fast and accurately.

The microwave power used to make the measurements is not the power used for irradiation. A separate source is used for that purpose, and the irradiating power is directed toward the sample before it is introduced in the cavity.

DISCUSSION ON
INTRODUCTORY SESSION INCLUDING SPECIAL TOPICS IN MICROWAVE MEASUREMENTS
AT THE CONFERENCE, 6TH SEPTEMBER, 1961

Mr. R. J. Meredith: In their paper, Drs. Bosch and Gambling describe a direct-detection spectrum analyser for measuring a.m. noise. It may be of interest that, at lower modulator frequencies, we have used similar systems using a direct-detection receiver and also a superheterodyne receiver system. In the latter we used balanced silicon mixer crystals at X band, the i.f. amplifier being followed by an envelope detector and spectrum analyser. This had a noise figure such that it should have been possible to measure noise sidebands of − 133 dB relative to carrier (measuring bandwidth, 100 c/s). In practice we found that a form of flicker noise occurred in the mixers which resulted in their conversion loss being noise-modulated. Thus a 'clean' input carrier became modulated with noise at low frequencies. The effect was sufficient to cause the receiver noise factor to be degraded by 10 dB or more below 5 kc/s. In the absence of an input signal the noise performance was normal. It seemed likely that the direct detection system also suffered from the same phenomenon.

It was not possible, therefore, to increase the sensitivity by increasing the input signal level because the mixer noise was increased in the same proportion.

Mr. I. A. Harris: Mr. Larson mentions the use of type-N coaxial connectors for frequencies up to 6 Gc/s. Are these the ordinary commercial N-type connectors or are they specially-made precise connectors which will mate with ordinary connectors? With a coaxial connector having the dimensions of the N-type, a tolerance of less than 0·001 in on the inner and outer conductor diameters is needed to be certain that the characteristic impedance is within 1% of nominal, such as would be required in moderately accurate impedance and power measurements.

Prof. A. L. Cullen: With regard to Mr. Rzymowski's paper,

is there any significant loss of accuracy in using these techniques compared with the standard standing-wave-indicator scales in which a vernier is usually available for measuring the position of the probe more accurately? Often, in measuring very small standing-wave ratios ($s < 1$) one wishes to use the von Hippel technique of measuring the width of the minimum. Are these scales and indicating mechanisms sufficiently accurate to enable this to be done?

Mr. J. A. Lane: I would like to have some comments from Mr. Larson on the facilities at the Electronic Calibration Center. Those of us in the United Kingdom who have been concerned with some aspects of this problem have two points very much in mind: first, the magnitude of the effort which is required to establish facilities of the comprehensive kind which Mr. Larson has described. In addition, we have to assess the relative importance of all the various parameters which apparently require standardization in the microwave field—power, impedance, attenuation, noise, Q-factor, etc. Can he comment on the extent to which the services in the Calibration Center are used by industry and the military services, and secondly how is the effort in the Calibration Center related to the equally important problem of maintaining an adequate research programme on the development of new techniques?

Drs. T. Sárkány and T. Bercell: We have been concerned with measurements on travelling-wave-tube noise at the Research Institute for Telecommunication in Budapest, Hungary. Mr. Burke has spoken about troubles arising from the high level of the carrier when measuring the noise sidebands, and he used a cancellation method for suppressing the carrier. We used different methods because we found the microwave method of cancellation was very sensitive to phase and amplitude changes. In one of our methods we also used a microwave spectrum analyser as Mr. Burke did, but instead of microwave cancellation we used the method of cutting off the i.f. amplifier of the analyser during the time the carrier was scanned by the local oscillator. The alternative method which we found useful was applying a base-band spectrum analyser scanning the output video signal of a broad-band microwave receiver in the range 50 kc/s–10 Mc/s. Owing to the a.c. coupling of the base-band amplifier of the microwave receiver used, we naturally had no output due to the carrier, and noise sidebands down to 70 or 80 dB could easily be measured in this way. We found the main frequency components of ion-modulation noise situated in the band 2–3 Mc/s.

Dr. W. A. Gambling: At Southampton University we have investigated the effects of positive ions in a backward-wave oscillator of special construction in which grids are located alongside the twin electron beams. The positive ions can be removed as soon as they are formed by the application of a negative voltage to these grids. It is found that positive ions can (*a*) cause discrete oscillations at sideband frequencies corresponding to the ion plasma frequency, (*b*) produce rather broad noise peaks covering a range of sideband frequencies of several kilocycles, and (*c*) increase the noise level over the entire sideband spectrum up to 100 Mc/s or more. Furthermore, in the absence of positive ions, even quite small amounts of power-supply ripple can increase the noise level appreciably. For example, it has been found that a ripple voltage of 10 μV at 1·5 Mc/s applied to the cathode of a 723 A/B klystron raises the noise level by 1 dB at sideband frequencies up to 100 Mc/s. I should like to ask Mr. Burke whether he has observed similar effects with travelling-wave tubes.

Another point is that the superheterodyne system measures 'total' noise, which is composed of background noise, a.m. noise and f.m. noise. Whether or not the total noise is a useful quantity depends on the system in which a particular device is to be used. For example, the f.m. noise of a local oscillator does not contribute to the noise factor of a receiver, so that, in this case, a measurement of total noise is not particularly useful. I mention this because by separating the various noise components of microwave oscillators we find that the f.m. noise can exceed the a.m. noise by as much as 20 dB. Has Mr. Burke observed a comparable effect in travelling-wave tubes operating non-linearly?

Prof. H. E. M. Barlow: I am interested in the power level of 10 mW/cm^2, which is said to be the threshold of safety for a person in the path of an electromagnetic wave, and I wonder whether Prof. Süsskind can tell us a little more about the considerations which led to the acceptance of this criterion. I gather that the figure quoted is applicable to practically the whole of the r.f. spectrum, and I wonder how far it is based on information about the behaviour of the body in regard to its absorbing and reflecting properties at different frequencies. It seems that, if a person goes into an intense electromagnetic field, then, in some circumstances, very strong standing waves are likely to be set up by the presence of his body, bringing about a complete change in both the field and the power distribution as compared with the original conditions. I think, therefore, that we must be quite clear what we require to measure. Do we want to know the power that is actually absorbed by the body as it moves about in the field, or do we want to measure the electric field incident on the body in its various locations? What precisely is the information required to ensure that there is no danger to a person placed in such a field, and do the requirements change over the frequency range under consideration?

Mr. A. Watson: I have been doing some experiments on the destruction of dry rot in woodwork by irradiating the walls and timber, and I would like to ask Prof. Süsskind whether he knows any device for measuring temperatures while the power is switched on. If a thermocouple is in the field it picks up something from the electric vector; a mercury thermometer is not acceptable, etc. At present we have to switch the power off before inserting the temperature-measuring device, which is not very satisfactory.

Mr. J. S. Williams: I want to carry out measurements with low-power magnetrons, mainly to demonstrate the characteristics of the magnetrons themselves rather than to use them as sources for supplying microwave power to other apparatus. I have approached various firms about this, and apparently it is not much done in this country, since most microwave test benches use reflex klystrons as oscillators. One or two firms have agreed to tender for magnetron equipment, and it appears that other colleges and universities are interested. In view of the possible biological hazards, I would be glad of information on any special precautions taken when using magnetrons. I am envisaging very-low-power devices, and certainly when working with magnetrons on low-power radar sets during the war my colleagues and I appear to have suffered no ill effects. However, I must consider the safety of students and other members of staff. I would therefore like to know whether anyone has had any appreciable experience or worry in this field, and whether commercial devices exist which will enable one to make an approximate but reasonably quick estimate of radiation within a laboratory. Even if one is using comparatively low-power magnetrons, concentrations of energy may occur in various parts of the laboratory owing to reflections from walls, etc., and a person working in such a place for a comparatively long period might receive injuries which would not occur with a more even distribution.

Mr. L. Bounds: Many of us have been making measurements with microwave power at levels well above the named safe limit, and normally precautions are taken, despite the fact that there

has so far been no recognizable damage accruing from exposure. Low-power magnetrons giving outputs of 10–200 W. which might be used in academic work, can be considered hazardous. In industry precautions are always taken, such as instructions not to look into apertures in the magnetron or the circuit, and coverings set around in the form of attenuating shields to avoid reflection of radiated energy from furniture and equipment in the vicinity. This sort of precaution is taken as a matter of course.

On the question of measuring the fields, and whether equipment is available, there are several instruments sold commercially, all I think imported into this country. They are calibrated as indicators of radiation above or below the recognized safety level. I believe that the actual response at the ends of the scale is 10–20 times the safe level.

The neon tuning indicator type 4662, pre-ionized by connecting to the lighting supply via a 100 kΩ resistor glows when exposed in an electromagnet field of intensity very near the accepted safe value.

Prof. H. E. M. Barlow: In our academic experience of microwave experiments at high power densities we have had no serious problems in avoiding danger to individuals operating such equipment. The eye is particularly sensitive to microwave radiation, and it is important not to look down the end of a waveguide working at high power, as for example one might be tempted to do when observing wave patterns displayed by neon discharge tubes fitted inside the guide. Provided that one keeps away from known concentrations of radiation, Mr. Williams can rest assured that there will be no danger.

Mr. R. E. Larson (*in reply*): Mr. Harris's question refers to the statement 'for components utilizing standard ¼ in coaxial terminals with standard N-type connectors, calibrations will be made over the frequency range of 300 to approximately 6000 Mc/s where feasible with the quantity being measured'. At present, this statement applies only to the measurement of attenuation of coaxial components having N-type connectors. Calibration systems for the measurement of power or impedance using coaxial transmission lines have not yet been established at the Electronic Calibration Center.

In connection with attenuation measurements in coaxial systems at the Center, standard commercially manufactured N-type connectors are used both for the terminals of the calibration system and the terminals of the inter-laboratory attenuation standards being calibrated. This is feasible for the following reasons. Commercially manufactured N-type connectors are made to military specifications (MS 91236 and MS 91237), which call for a tolerance of ±0·001 in on the outside diameter of the inner conductor and a tolerance of ±0·002 in on the inside diameter of the outer conductor. Before making an attenuation measurement with the present system, the system terminals are matched, by adjusting appropriate 'tuners' placed immediately behind each terminal, to a v.s.w.r. of better than 1·10. The v.s.w.r. of the terminals of the inter-laboratory standard is also measured. The maximum limit of error due to mismatch can then be calculated. It emerges that more difficulty is encountered with failure of connectors to mate properly because of poor assembly of parts and attention to proper placement along the axis of the connector. Critical dimensions of the connectors have been established and are required by the Center for attenuation measurements with coaxial systems.

The first part of Mr. Lane's question can be answered by giving some brief data on the relative use made of the services of the Electronic Calibration Center in the microwave region. From July, 1959, to June, 1960, the distribution of calibration work performed for industry and the military services was, respectively, about 35 and 65%. The same approximate percentages obtain when the comparison is made on the basis of number of items calibrated or on the basis of man-hours required to perform the calibrations. From July, 1960, to June, 1961, the distribution of calibration work performed for industry and the military services was, respectively, about 20 and 80%; and from July, 1961, to October, 1961, similar data reveal the respective distribution to be about 45 and 55%. The reasons for the variation in the distribution of calibration work probably include factors which are very difficult to appraise. There is a close relationship between many industrial activities and military interests through the military contracts made with industrial concerns.

The second part of Mr. Lane's question deals with the relationship between the satisfactory performance of calibrations and an adequate research programme on the development of new measurement techniques. There is an obvious relationship here, which is recognized by Mr. Lane. There are constant and continuing demands for more calibration capabilities by the Electronic Calibration Center. This demand from the military services was perhaps the outstanding factor which led to the establishment of the Electronic Calibration Center within the framework of the National Bureau of Standards. There has since developed a perhaps equally pressing demand from industry in the United States for the development of these capabilities.

One of the basic missions of the N.B.S. includes the development of more microwave measurement techniques and the continual improvement of those already begun. The Bureau's support of the development of microwave measurement techniques existed before the establishment of the Center, and it has since been strengthened. In the microwave region and other frequency ranges, the efforts of the Center are also added to this development work. I believe that these statements are borne out by the presentation of papers at the Conference from the N.B.S. It is of interest that in the microwave section of the Center approximately 50% of the manpower is presently devoted to the development of new and improved measurement techniques, while the remaining effort is applied to the calibration of inter-laboratory standards, maintenance of present calibration equipment, construction of additional calibration systems, and other duties.

Mr. E. Rzymowski (*in reply*): In reply to Prof. Cullen, we have to consider separately the accuracy of the readings using 'scales' and 'phase-angle reading mechanisms'.

Scales calibrated in fractions of a guide wavelength are divided every $0{\cdot}01\lambda_g$, and estimations to $0{\cdot}002\lambda_g$ can easily be made by eye. This accuracy compares favourably with that of readings made with a scale graduated in millimetres, but probably it is somewhat inferior to what can be achieved using a well-made scale and vernier.

A clock gauge is used for reading fractions of a guide wavelength in phase-reading mechanisms. Then $0{\cdot}001\lambda_g$ is read directly, and further subdivision is possible. The accuracy of this mechanism is superior to that of a scale and vernier and equal to that achieved with a clock gauge attached directly to a standing-wave-meter carriage, only much quicker results are obtained.

Drs. B. G. Bosch and **W. A. Gambling** (*in reply*): We would confirm Mr. Meredith's observation that flicker or excess noise in the mixer crystal is the factor which limits the sensitivity of a microwave noise-measuring equipment. At low frequencies the excess noise power of a crystal is inversely proportional to frequency,* and below 5 kc/s the noise-temperature ratio is likely to be greater than 20 dB. In a direct-detection system

* Bosch, B. G., Gambling, W. A., and Wilmshurst, T. H.: 'Excess Noise in Microwave Mixer Crystals', *Proceedings of the Institute of Radio Engineers*, 1961, 49, p. 1226.

the only method of reducing the effect of crystal noise might be to precede the mixer by a linear low-noise microwave amplifier such as a travelling-wave tube. The noise-temperature ratio varies considerably, particularly at low frequencies, even among crystals of the same type,* and for the highest sensitivity a large number of crystals should be tested before a choice is made. In a superheterodyne system the noise from the local oscillator, if troublesome, can be suppressed by up to about 50 dB in a balanced mixer. The effect of excess noise in the mixer crystal(s) can be reduced by using a high intermediate frequency, but the penalty to be paid is the necessity for an increased stability of the test and local-oscillator frequencies. For example, if it is desired to measure accurately the noise level 5 kc/s from the test-oscillator carrier frequency, the test and local-oscillator frequencies must drift relative to each other (and preferably absolutely) by appreciably less than 500 c/s. At microwave frequencies this is not easy and usually requires locking of the two oscillators. Further difficulties are then produced as the noise properties of the locked oscillator can be changed by the locking process if care is not taken.

Prof. C. Süsskind (*in reply*): In reply to Prof. Barlow, most irradiations carried out for the purpose of measuring biological effects take place in free space, in properly terminated waveguides, or in anechoic chambers, so that various investigators may compare their findings, based on the incident power density before any body is inserted. Under these conditions, noticeable effects (such as temperature rise) begin to occur at microwave frequencies at average c.w. densities of the order of 100 mW/cm^2; the figure varies with frequency and surface conditions, largely owing to changes in the distance to which the radiation penetrates. It is recognized that internal and external standing waves may be actually set up after the body is inserted or owing to waves reflected from nearby structures under practical conditions. This is one of the reasons why a safety factor of not less than 10 is employed in setting the criterion at 10 mW/cm^2. Conditions at the body surface, which are particularly sensitive to frequency changes, are perhaps even more significant, as Prof. Schwan at the University of Pennsylvania has repeatedly pointed out.† It would doubtless be better if a measure of the absorbed power could be obtained. Various calorimetric methods have been proposed, including a box of jelly with electrical properties approximating to those of the human body and containing a temperature-sensing element. None has proved wholly satisfactory; nor have the problems of varying surface conditions and internal standing waves been solved. One of the reasons is that the measuring device inserted in the irradiated body or simulating it often tends to perturb the field it measures, which is perhaps another point in favour of making comparative measurements in the free field, where such perturbations may be minimized.

In this connection, and in answer to Mr. Watson, it should be pointed out that we have nevertheless had good luck with thermistors made for biological purposes. In measuring rectal temperatures of mice, for instance, we have used thermistors with sensing elements embedded in glass tubing only 0·100 in in diameter, and I believe that even smaller units are available. By suitable orientation of the device with regard to the electric-field vector and by shielding the lead wires in absorbent material, we were able to reduce direct microwave pick-up to such an extent that the spurious signal represented only a small fraction of the thermistor output voltage, a fraction for which we could readily compensate on the automatic data plotter. Calibration against other methods of measuring temperatures showed that no perceptible increase resulted locally from the presence of the thermistor tip, at the low power level employed. I cannot say whether this method would answer in the application proposed here, which is probably characterized by higher powers and lower temperature conductivities.

Mr. P. F. C. Burke: The methods of noise sideband measurement described by Drs. Sárkány and Berceli are certainly satisfactory but require one to attenuate the output of the travelling-wave tube from about +37 dBm to less than −10 dBm in order to avoid non-linearity in the crystal mixer. To distinguish between a.m. and f.m. noise, some form of video receiver following the appropriate detector is necessary, as Dr. Gambling points out. The method described in the paper is adequate for checks on the presence of plasma noise and its correlation with tube conditions. We have not yet made as extensive a study of this phenomenon in travelling-wave amplifiers as Dr. Gambling has in backward-wave oscillators. I would agree with his findings, except that we have not noticed large amounts of noise arising from supply-voltage ripple as he describes.

* Bosch, B. G., Gambling, W. A., and Wilmshurst, T. H.: 'Excess Noise in Microwave Mixer Crystals', *Proceedings of the Institute of Radio Engineers*, 1961, 49, p. 1226.

† *Proceedings of the Institute of Radio Engineers*, 1956, 44, p. 1572; 1959, 47, p. 1841.

REFERENCES

1. I. W. Sizer, in "Advances in Enzymology" (F. Nord and C. Werkman, eds.), Interscience Publishers, New York, 1943; vol. 3, p. 35.
2. G. Z. Ammann, Physk. Chem. 18: 426, 1895.
3. M. Dixon and E. C. Webb, "Enzymes," Academic Press, New York, 1958; p. 152.
4. P. F. Fenton and G. R. Cowgill, in "Textbook of Physiology" (J. Fulton, ed.), W. B. Saunders Co., Philadelphia and London, 1949; p. 927.
5. P. Green and E. Wade, Canad. Med. Assoc. J. 66: 175, 1952.
6. T. Jaski and C. Susskind, Science 133: 443, 1961.
7. P. O. Vogelhut, "The Dielectric Properties of Water and Their Role in Enzyme-Substrate Interactions," University of California (Electronics Research Laboratory), Series 60, Issue No. 476, August 24, 1962.

DISTRIBUTION LIST

CONTRACT NO. AF 61(657)-114

ADDRESSEE	NO. COPIES
RADC (RASH1, ATTN: W J. Doherty) Griffiss AFB, New York	4
RADC (RAAPT) Griffiss AFB, New York	1
RADC (RAALD) Griffiss AFB, New York	1
GEEIA (ROZMCAT) Griffiss AFB New York	1
RADC (RAIS, ATTN: Mr. Malloy) Griffiss AFB New York	1
U S Army Electronics R & D Labs Liaison Officer RADC Griffiss AFB, New York	1
AUL (3T) Maxwell AFB, Alabama	1
ASD (ASAPRD) Wright-Patterson AFB, Ohio	1
Chief, Naval Research Lab ATTN: Code 2027 Washington 25, D C	1
Air Force Field Representative Naval Research Lab ATTN: Code 1010 Washington 25, D C	1
Commanding Officer U S. Army Electronics R & D Labs ATTN. SELRA/SL-ADT Fort Monmouth, New Jersey	1
National Aeronautics and Space Administration Langley Research Center Langley Station Hampton, Virginia ATTN: Librarian	1
Central Intelligence Agency ATTN: OCR Mail Room 2430 E Street NW Washington 25, D C	1
U.S. Strike Command ATTN: STRJ5-OR Mac Dill AFB, Florida	1
AFSC (SCSL) Andrews AFB Washington 25, D C	1
Commanding General U S Army Electronic Proving Ground ATTN: Technical Documents Library Fort Huachuca, Arizona	1
ASTIA (TISIA-2) Arlington Hall Station Arlington 12, Virginia	10

ADDRESSEE	NO COPIES
AFSC (SCFRE) Andrews AFB Washington 25, D C	1
Hq USAF (AFCOA) Washington 25, D. C.	1
AFOSR (SRAS/Dr. G R. Eber) Holloman AFB, New Mexico	1
Office of Chief of Naval Operations (Op-724) Navy Dept Washington 25, D C.	1
Commander U S Naval Air Development Center (NADC Library) Johnsville, Pennsylvania	1
Commander Naval Missile Center Technical Library (Code NO 3022) Point Mugu, California	1
Bureau of Naval Weapons Main Navy Bldg Washington 25, D. C. ATTN: Technical Librarian, DLI-3	1
Redstone Scientific Information Center U S. Army Missile Command Redstone Arsenal, Alabama	1
Commandant Armed Forces Staff College (Library) Norfolk 11, Virginia	1
ADC (ADOAC-DL) Ent AFB, Colorado	1
AFFTC (FTOOT) Edwards AFB, California	1
Commander U S. Naval Ordnance Lab (Technical Library) White Oak, Silver Springs, Maryland	1
Commanding General White Sands Missile Range New Mexico ATTN. Technical Library	1
Director U S Army Engineer R & D Labs Technical Documents Center Fort Belvoir, Virginia	1
ESD (ESRL) Laurence G Hanscom Field Bedford, Massachusetts	1
Commanding Officer and Director U S. Navy Electronics Lab (LIB) San Diego 52, California	1
ESD (ESAT) Laurence G Hanscom Field Bedford Massachusetts	1

ADDRESSEE	NO COPIES
Commandant U.S Army War College (Library) Carlisle Barracks, Pennsylvania	1
APGC (PGAPI) Eglin AFB, Florida	1
AFSWC (SWOI) Kirtland AFB, New Mexico	1
AFMTC (Technical Library MU-135) Patrick AFB, Florida	1
RTD (RTGS) Bolling AFB Washington 25, D C	1
Chief Research & Development Office of Chief Signal Officer Washington 25, D. C.	1
School of Aerospace Medicine Col. John E. Pickering Brooks AFB, Texas	1
Dr. Frank Hartman Hq USAF (AFCSG) Washington 25, D.C.	1
Colonel G. M Knauf Directorate of Aerospace Medicine Hq NASA 801 19th Street, NW Washington 25, D C	1
Lt. Col. L C MacMurray SGO, Main Navy Building Washington 25, D C	1
Armed Forces Institute of Pathology Washington 25, D. C	1
Dr R A Weale Institute of Ophthalmology University of London Judd Street London, W C 1, England	1
Herbert S Brownstein Directorate of Aerospace Medicine Hq NASA 801 19th Street, NW Washington 25, D C	1
For C-E Equipment Only	
European GEEIA Rgn (ZEM) APO 332 New York, New York	1
Hq Pacific GEEIA Rgn (ZPM) APO 958 San Francisco California	1
Western GEEIA Rgn (ZSM) McClellan AFB, California	1
Eastern GEEIA Rgn (ZMMRS) Brookley AFB, Alabama	1
Inspection Office (Technical Library) Central GEEIA Rgn (AFLC) Tinker AFB, Oklahoma	1

DISTRIBUTION LIST

CONTRACT NO Nonr-222(92)

Office of Naval Research Biochemistry Branch (Code 442) Washington 25, D C	1
Director, Naval Research Laboratory ATTN: Technical Information Officer Washington 20, D. C.	6
Office of Technical Services Department of Commerce Washington 25, D C	1
Armed Services Technical Information Agency Documents Service Center Arlington Hall Station Arlington 12, Virginia	5
Office of Naval Research Branch Office Tenth Floor The John Crerar Library Building 86 East Randolph Street Chicago 1, Illinois	1
Office of Naval Research Branch Office 346 Broadway New York 13, New York	1
Office of Naval Research Branch Office 930 East Green Street Pasadena 1, California	1
Director Aviation Medical Acceleration Laboratory U.S Naval Air Development Center Johnsville, Pennsylvania	1
Superintendent Aeronautical Medical Equipment Laboratory U.S. Naval Air Experimental Station Naval Air Material Center Naval Base Philadelphia, Pennsylvania	1
Commander Naval Air Test Center Aero Medical Branch of Service Test Patuxent River Maryland	1
Office of Naval Research Branch Office 1000 Geary Street San Francisco 9, California	1

U S Navy Office of Naval Research Branch Office, Box 39 Navy No 100 Fleet Post Office New York, New York	2
Director, Research Division Bureau of Medicine & Surgery Department of the Navy Washington 25, D C	2
Headquarters U S. Air Force Washington 25, D C. ATTN AFDRD -- HF	1
Office of the Surgeon General Department of the Army Washington 25 D C	1
National Research Council Division of Medical Sciences 2101 Constitution Avenue, NW Washington 25, D C	1
Commanding Officer Naval Medical Research Institute National Naval Medical Center Bethesda 14, Maryland	2
The Director Armed Forces Medical Library 7th Street and Independence Avenue, SW Washington 25, D.C.	1
Commanding Officer U S Naval Unit Biological Division Chemical Corps Camp Detrick Frederick, Maryland	1
Radiological Medical Director U.S. Naval Radiological Defense Laboratory Naval Supply Depot San Francisco 24, California	1
Head, Medical Research Department U.S. Navy Mine Defense Laboratory Panama City, Florida	1

Commanding Officer U S. Naval Medical Research Unit No. 1 Building T-19 University of California Berkeley 4, California	1
Commanding Officer U.S Naval Medical Research Unit No. 2 APO 63 San Francisco, California	1
Commanding Officer U S. Naval Medical Research Unit No. 3 Box "E" -- APO 231 c/o Postmaster New York City, New York	1
Officer in Charge U S. Naval Medical Research Unit No. 4 Naval Training Center Great Lakes, Illinois	1
Officer in Charge U S Naval Medical Research Laboratory Submarine Base New London, Connecticut	1
Commanding Officer U.S. Naval Medical Field Research Laboratory Camp Lejeune, North Carolina	1
Commanding Officer U.S Naval School of Aviation Medicine Naval Air Station Pensacola, Florida	1
Chief of Medicine and Research Director Metabolic Research Facility U S. Naval Hospital Oakland 14, California	1
Chief, Amputee Service Navy Prosthetic Research Laboratory U.S. Naval Hospital Oakland 14, California	1
Research and Development Division Office of the Chief Signal Officer Department of the Army Washington 25, D C.	1

EFFECTS OF CHRONIC MICROWAVE IRRADIATION ON MICE

BY

S. PRAUSNITZ AND C. SÜSSKIND

Reprinted from IRE TRANSACTIONS
ON *BIO-MEDICAL ELECTRONICS*
Volume BME-9, Number 2, April, 1962

PRINTED IN THE U.S.A.

EFFECTS OF CHRONIC MICROWAVE IRRADIATION ON MICE

BY

S. PRAUSNITZ AND C. SÜSSKIND

Reprinted from IRE TRANSACTIONS
ON *BIO-MEDICAL ELECTRONICS*
Volume BME-9, Number 2, April, 1962

PRINTED IN THE U.S.A.

Effects of Chronic Microwave Irradiation on Mice*

S. PRAUSNITZ† AND C. SÜSSKIND†, SENIOR MEMBER, IRE

Summary—**An experiment has been carried out to determine pathological and longevity effects caused by chronic microwave irradiation of mice. Two hundred males were exposed daily for 59 weeks to 0.100 w/cm² for 4.5 minutes. This treatment produced an average body temperature rise of 3.3°C. Histopathology was performed on all dead mice in both irradiated and control group. Changes in body weight, in body temperature response to heating, and in the blood picture were not evident. Testicular degeneration in the form of tubule atrophy and neoplasms of the white cells were indicated. Longevity of the mice did not appear to be affected under the prevailing conditions.**

THE ACUTE EFFECTS of microwave radiation on experimental animals have been the object of numerous studies during the past few years. Whole-body exposure has been shown by Süsskind *et al.*[1] and Ely and Goldman[2] to result either in death within 24 hours or survival with no apparent permanent damage. Carpenter[3] and others have reported that local exposure of the eye may result in lenticular opacities, although all studies to date involving whole-body irradiation have shown negative results with regard to ocular effects. Carpenter[3] has also shown that opacities may result as a cumulative effect of repeated subthreshold exposures. Imig, Thomson, and Hines[4] reported that irradiation of the scrotum can lead to testicular degeneration. Other effects, of a temporary nature, such as superficial burning (Howland and Michaelson)[5] and alterations in the blood picture (Deichmann, Stephens, Keplinger, and Lampe)[6] have also been reported.

It was the purpose of the present investigation to determine the pathological and longevity effects of chronic whole-body microwave radiation at 3 cm, since no definitive study of this nature has been reported to date.

Calibration Procedures

Electromagnetic

The microwave calibration was carried out with particular care since it was felt that the measurement of absolute microwave power was the weak point in many other investigations in this field. The calibration procedure has been described in considerable detail previously.[1] A brief résumé follows.

The radiator used throughout was a standard horn with the following dimensions: apex-to-aperture distance, 17.7 cm; aperture, 4 × 6 cm; length of horn edge, 10.3 cm. The generator was a government-furnished AN/TPS-10D radar transmitter capable of delivering 25 w (average) at 9270 Mc in 2-μsec pulses with a pulse repetition frequency of 500 pps (*i.e.*, a duty cycle of 0.001).

For an order-of-magnitude calibration, a resonant $\lambda/2$ dipole loaded by a neon bulb was developed; the light output, guided along a Lucite rod to a photomultiplier tube, was transformed to a voltmeter reading calibrated in terms of the known output of the source at a given distance. The final reading concerns the total power radiated, obtained by integration of measurements taken over a large area (1 sq ft) containing the experimental point (at a distance, along the axis, of 12 λ from the apex of the radiator horn). This calibration yielded a power density of 0.16 w/cm² at the experimental point when the source operated at the full 25-w level.

A more accurate determination was attempted by means of a precision-built resonant $\lambda/2$ dipole connected to a thermistor via a balun line, a Microdot co-ax, and a precision attenuator. This measurement likewise yielded the value of 0.16 w/cm², indicating that the test probe was not limiting the accuracy of the calibration.

Because this value of power density would be insufficient for some of the projected experiments, the animals would have to be placed even nearer to the radiator, with the calibration difficulties of working in the near zone of the antenna. A specially constructed range equipment was employed for the purpose of taking E and H field patterns over ±145° from the axis at increasing distances. This "three-dimensional" plot of the radiation pattern was then integrated numerically and equated to the total radiated power, measured separately by calorimetric methods. This series of measurement yielded the corrected value of 0.146 w/cm² at the experimental point; this value was made the basis of the over-all calibration.

The irradiations were carried out in a walk-in anechoic

* Received by the PGBME, October 30, 1961. This investigation was supported by the U. S. Air Force, Contract AF41(657)-114. Some of the results reported herein were presented at the 4th International Conference on Medical Electronics, New York, N.Y., 1961, and appear also in C. Süsskind and Staff, "Longevity Study of the Effects of 3-cm Microwave Radiation on Mice," Electronics Research Laboratory, University of California, Ser. 60, Issue No. 382; June, 1961.

† Electronics Research Laboratory, University of California, Berkeley, Calif.

[1] C. Süsskind and Staff, "Biological Effects of Microwave Radiation," Electronics Res. Lab., University of California, Berkeley, Ser. 60, Issue No. 205; June, 1958.

[2] T. S. Ely and D. E. Goldman, "Heating Characteristics of Laboratory Animals Exposed to 10-cm Microwaves," Naval Med. Res. Inst., Bethesda, Md., Res. Rept., Project NM001, p. 84; 1957.

[3] R. L. Carpenter, D. K. Biddle, and C. A. Van Ummersen, "Opacities in the lens of the eye experimentally induced by exposure to microwave radiation," IRE TRANS. ON MEDICAL ELECTRONICS, vol. ME-7, pp. 152–157; January, 1960.

[4] C. J. Imig, J. D. Thomson, and H. M. Hines, "Testicular degeneration as a result of microwave irradiation," *Proc. Soc. Exptl. Biol. Med.*, vol. 69, pp. 382–386; 1948.

[5] J. W. Howland and S. Michaelson, "Studies on the biological effects of microwave irradiation of the dog and rabbit," *Proc. 3rd Annual Tri-Service Conf. Biol. Effects of Microwave Radiating Equipments*, Berkeley, Calif., pp. 191–238; August, 1959.

[6] W. E. Deichmann, F. H. Stephens, Jr., M. Keplinger, and K. F. Lampe, "Acute effects of microwave radiation on experimental animals (24,000 Mc)," *J. Occupational Med.*, vol. 1, pp. 369–381; 1959.

chamber constructed for the purpose, lined on all sides by broad-band absorbent material of the animal-hair type intended for use at X band and higher frequencies. Single mice in plastic-net enclosures were used for body-temperature calibrations, and cages containing 10 animals were used for the irradiation series itself, as described below. The effects of RF energy on the thermistor thermometers and the associated leads were minimized by suitable orientation of these components; the remaining effects were carefully compensated for in evaluating the results.

Body temperature was measured rectally by means of a small glass-covered Veco thermistor with an OD of 0.100 in inserted to a distance of 0.5 in. The thermistor formed one arm of a dc unbalanced bridge, the output of which was connected to a Varian Model G-10 adjustable-span graphic recorder. The system was capable of resolving temperature readings of 0.1°C; the limit of error of the system was 1 per cent of span or 0.1°C.

Biological

In order to make possible the choice of a suitable dose for chronic irradiation a preliminary study was carried out in which acute lethal doses at power-density levels between 0.068 and 0.380 w/cm² were determined. Individual male mice were exposed to 3.2-cm microwaves for whole-body irradiation (with ventral exposure) during which time rectal temperatures were recorded. Whole-body exposure to microwave radiation caused a rise in the body temperature of the animal. Death could be correlated with the maximum body temperature reached: 50 per cent of the mice died if their body temperature reached 44.1°C, or 6.7°C above normal. (Treatment with chlorpromazine, which lowered body temperature by several degrees, permitted correspondingly larger temperature rises.) All deaths were found to occur within 24 hours of irradiation.

The survivors of the LD_{50} irradiation dose (a dose that kills 50 per cent of the irradiated mice) were bred as a rough check of testicular damage. All sired litters—indicating that a single exposure to a dose that killed half the animals did not render the survivors sterile.

The graphs in Fig. 1 show the average temperature rise per minute for the range 0.014 to 0.380 w/cm². The dashed horizontal line at $\Delta BT = 6.7°C$ indicates the temperature at which 50 per cent of the mice die. This line dips at the higher power densities to compensate for a rise in body temperature after the power is turned off. It may be seen from Fig. 1 that power densities below 0.068 w/cm² did not cause acute deaths. The dose depends upon both the power density and the length of exposure; the LD_{50} could be obtained, for example, by exposure to 0.100 w/cm² for 12 minutes or to 0.270 w/cm² for 3.75 minutes.

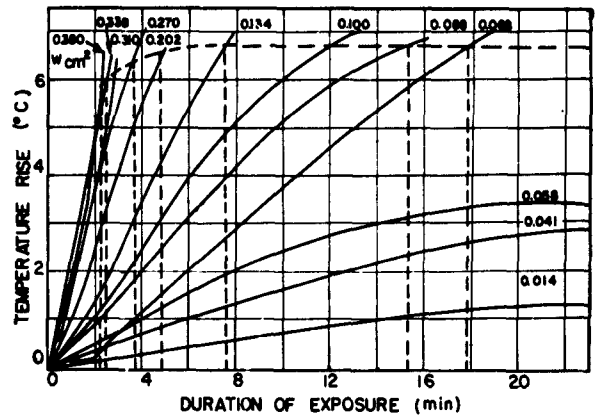


Fig. 1—Average temperature rise as a function of time at various power densities. Dashed lines indicate LD_{50} for the various densities.

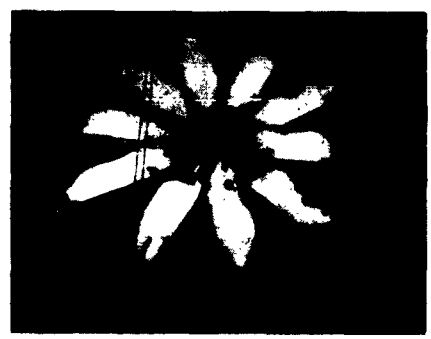


Fig. 2—Polystyrene cage used for group irradiations in the longevity experiment.

Longevity Experiment

Procedure. A colony of 300 male Swiss albino mice of identical age was housed in a cabinet, the temperature of which was maintained between 21 and 24°C. The cages were cleaned twice a week and the mice did not come into contact with any other animals. All mice were dipped into a solution containing Aramite and Nacconal every 3 months as protection against mites and were dusted periodically with Rothrum powder as a measure against lice. Terramycin was administered in the drinking water (2 tsp/220 cc of water) to fight secondary infection accompanying a small epidemic of pneumonitis. Each cage containing 10 mice constituted a unit; each unit was irradiated in a circular, compartmentalized polystyrene cage with a bottom made of plastic window screen (Fig. 2). The cage was suspended from above in a horizontal position and was irradiated from below. To minimize the effect of multiple reflections, especially between adjacent animals, the cage was rotated at 1 rpm during irradiations. The power drop across the width of the cage was no more than 1 db. Control animals were likewise placed into a rotating cage, but in a mock-up chamber at room temperature. None of the mice in these tests was pretreated with a tranquilizer or anaesthetic.

Two hundred mice were irradiated for 59 weeks, 5 days/week for 4.5 minutes at a power density of 0.100 w/cm². This was one-half the LD_{50}, since 9 minutes of irradiation at this power density causes 50 per cent of deaths. The average body temperature rise for this dose

was 3.3°C (2.0 to 5.0°C), which duplicated an interpolation of the preliminary results shown in Fig. 1. Body temperature response was recorded only periodically for 5 given mice, which were irradiated for this purpose individually without rotation.

When an animal died it was autopsied as soon as found and key tissues of those animals which had not undergone extensive post mortem changes were prepared for histological examination. The tissues studied included liver, spleen, lymph nodes, kidneys, adrenals, gut, lungs, and testes. Other tests performed on both groups of mice included random blood counts, spot checks of urine for glucose, weekly weighing of all mice, recording of body temperature response of 5 given mice, and three sacrifice series to assess the condition of surviving animals.

Results. The rate at which the mice in both irradiated and control groups died is shown in Fig. 3. As is indicated, the rate of lethality could not be correlated with the irradiated group and was in fact somewhat higher in the control group. Part of the colony suffered from pneumonia during the last 3 months of irradiations; this factor was responsible for a number of deaths in both groups during that period. (Conceivably microwave irradiation in this modality, with periodically induced slight artificial fevers, is of some benefit to the animal in combatting disease.)

Blood counts were made of a random 10 per cent of both groups after four months of irradiation. Blood samples were taken from the tail. A few mice, which subsequently died, showed white blood cell counts ranging between 77,000 and 125,000 cells/mm³. Autopsy of these mice revealed enlargement of lymphoid tissue and what appeared to be enormous liver abscesses. The rest of the mice had counts within normal limits (Table I).

All spot checks of the urine to detect the presence of glucose were negative.

The body-temperature rise of 5 given mice was periodically checked in order to determine whether this response changed as the experiment progressed. As may be seen in Fig. 4 this response appeared to remain quite constant: the fluctuation did not follow a particular trend.

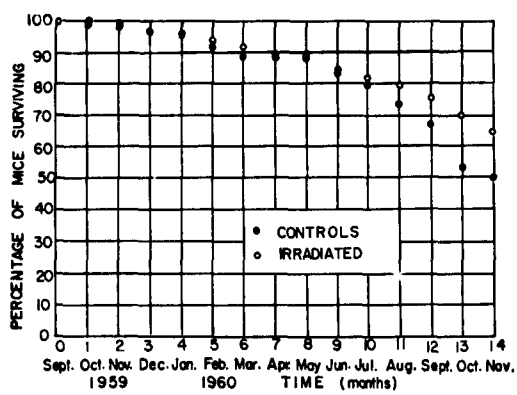


Fig. 3—Percentage of 200 irradiated and 100 control mice surviving daily sublethal irradiations. At the start of the experiment, mice were 3 months old.

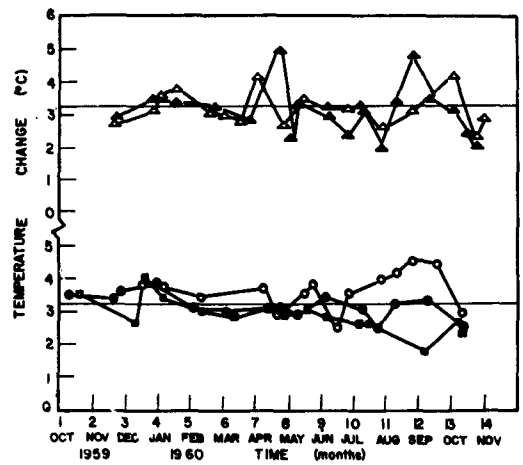


Fig. 4—Maximum temperatures reached during irradiation of 5 mice.

Each unit of mice was weighed weekly and the average mouse body weight was computed (Fig. 5). The limits of the statistical spread of weights of the two groups overlap at each point. It thus appears that chronic irradiation of this type produces essentially no difference between the weight changes of irradiated and control mice. Nieset mentions in a preliminary report[7] a gain in weight for mice receiving chronic microwave irradiation; however, this result was evidently not borne out later (Baus and Fleming[8]). Moreover, the conditions were quite different from those prevailing in this Laboratory in that Nieset employed 10-cm microwaves, which he applied to the mice intermittently in order to prevent a temperature rise.

A compilation of the histological findings on autopsied mice which died during the course of the longevity experiment is shown in Table II. Although there was a total of 300 mice in the experiment, 132 were sacrificed in three sacrifice series performed to assess damage in surviving mice, and 68 dead mice had undergone extensive post

TABLE I

SUMMARY OF RED AND WHITE BLOOD COUNTS

	RBC (million cells/mm³)	WBC (cells/mm³)
Irradiated mice	9–13	16,000–31,000
Control mice	8–14	11,000–34,000*

* One mouse had a WBC of 7000 cells/mm³.

[7] R. T. Nieset *et al.*, "Investigations of the Biological Effects of Microwave Irradiation," Tulane University (Biophysics Program), New Orleans, La. Annual PR (Contract Nonr 47503); November, 1957.

[8] R. Baus and J. D. Fleming, "Biological effects of microwave radiation with limited body heating," *Proc. 3rd Annual Tri-Service Conf. Biol. Effects of Microwave Radiating Equipments*, Berkeley, Calif., pp. 291-313; August, 1959.

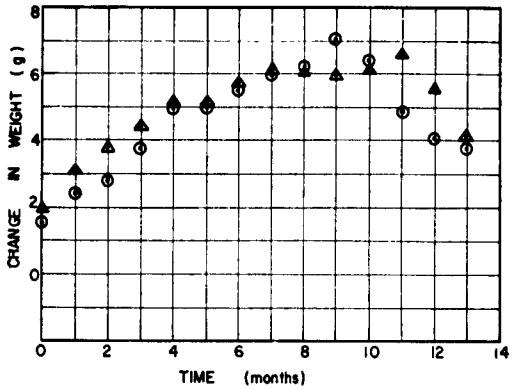


Fig. 5—Changes in weight in longevity experiment. Circles indicate irradiated animals; triangles, controls.

TABLE II

LONGEVITY ASSESSMENT

Condition	Number of Irradiated Mice in Which Condition Was Seen	Number of Control Mice in Which Condition Was Seen
Sterility	23 (40 per cent)*	3 (8 per cent)†
Leucosis	21 (35 per cent)*	4 (10 per cent)†
Lung Tumor	6	5
Thickened Arterioles		
Liver	2	0
Kidney	2	4
Lung	3	1
Spleen	1	0
Infection		
Mice with Pneumonia	26	18
No Pneumonia	9	16
Total Number of Samples	60	40
No examination of testis	3	3

* Per cent of total number of irradiated mice examined.
† Per cent of total number of control mice examined.

mortem changes that prohibited a meaningful histological assessment. The remaining 100 animals were used to compile Table II. The results indicate two conditions considerably more prevalent in the irradiated group than in the controls.

The first, testicular degeneration, was found in 40 per cent of the dead irradiated mice and in only 8 per cent of the controls. The testes were found to be atrophied and containing few or no sperm. The incidence of testicular degeneration is shown graphically in Fig. 6. Thirty irradiated and 30 control mice were bred with untreated females in order to study this phenomenon further. However, no litters appeared in either group, perhaps owing to the age of the males (1.5 yr) and the fact that they had never been bred previously.

The second pathological condition, found in 35 per cent of the irradiated mice and in 10 per cent of the controls, was cancer of the white cells. This condition manifested itself either as monocytic or lymphatic leucosis or lymphatic or myeloid leukemia. Leucosis is defined as a noncirculating neoplasm of the white cells, whereas leukemia is a circulating leucosis.

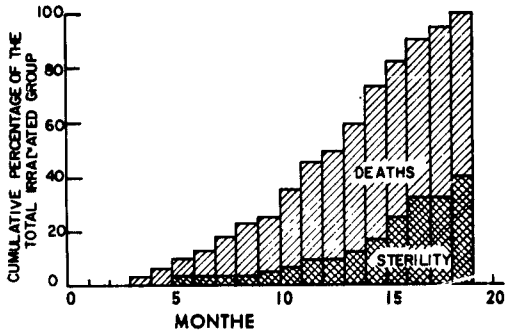


Fig. 6—Relation of testicular atrophy and death in the total examined irradiated group as a function of time.

Three sacrifice series were carried out 7, 16, and 19 months after the start of the longevity program. The second sacrifice series was performed within 1 month after the final irradiation; the third was executed 4 months after the final irradiation.

In the first series 5 per cent of both groups was sacrificed. Tail blood was taken from 10 irradiated and 5 control animals for total and differential white blood counts. The mice were then given a strong dose of ether and were autopsied. Tissues removed for histological examination were the adrenals, kidneys, liver, duodenum, spleen, testes, and brain.

On the basis of six conditions evident in a large proportion of the mice, criteria were set up for separating the controls from the irradiated. These criteria were:

1) Lymphoid infiltration in the brain, kidney, and/or duodenum.
2) Seroid deposits in the interstitial cells of the testes.
3) Presence of anomalous basophilic cells in the seminiferous tubules of the testes.
4) Absence of vacuoles in the adrenal cortex.
5) Presence of congestion in the kidney.
6) Results of total and differential white blood counts.

None of the above categories produced a correlation between the condition and irradiation.

The second sacrifice series, containing twice as many animals, was carried out in the same manner as the first, except that no blood or brain samples were taken. The only finding that was evident in this series was that 30 per cent of the irradiated and 10 per cent of the control mice had a leucosis, there were no correlations with regard to testicular degeneration, as in the acute-death histologies.

It was then decided that to resolve this partial contradiction, a third sacrifice series should be performed on the remainder of the mice: 67 irradiated and 19 controls. In this series testis and liver sections were examined microscopically for tubule atrophy and abdominal lymphomas,

LIST OF SCIENTIFIC REPORTS PUBLISHED UNDER CONTRACTS AF41(657)-114 AND Nonr-222(92)

Annual SR (1957–58): C. Süsskind and Staff, "Biological effects of microwave radiation," Series No. 60, Issue No. 205, June 30, 1958.

Annual SR (1958–59): C. Süsskind and Staff, "Cellular and longevity effects of microwave radiation," Series No. 60, Issue No. 241, June 30, 1959.

Annual SR (1959–60): C. Süsskind and Staff, "Microwave radiation as biological hazard and tool," Series 60, Issue No. 285, June 30, 1960.

Annual SR (1960–61): C. Süsskind and Staff, "Longevity study of the effects of 3-cm microwave radiation on mice," Series 60, Issue No. 382, June 30, 1961.

P. O. Vogelhut, "The dielectric properties of water and their role in enzyme-substrate interactions," Series 60, Issue No. 476, August 24, 1962.

Annual SR (1961–62) and FR (1957–62): C. Süsskind and Staff, "Nonthermal effects of microwave radiation," Series 60, Issue No. 489, October 31, 1962.

LIST OF PUBLICATIONS ARISING FROM WORK SUPPORTED FULLY OR IN PART BY CONTRACTS AF41(657)-114 AND Nonr-222(92)

B. S. Jacobson and C. Süsskind, "Effects of microwave irradiation on internal temperature and viability in mice," *Proc. Sec. Tri-Service Conf. Biol. Effects of Microwave Energy*, Rome, N.Y., July 8–10, 1958; pp. 234–239.

B. S. Jacobson, S. Prausnitz, and C. Süsskind, "Microwave radiation as a tool in biophysical research," *Institute of Radio Engineers National Convention Record*, 1959, vol. 7, Pt. 9, pp. 13–15.

B. S. Jacobson, S. B. Prausnitz, and C. Süsskind, "Investigation of thermal balance in mammals by means of microwave radiation," *Trans. Inst. Radio Eng.* (Prof. Gp. on Medical Electronics) ME-6: 66–68, 1959.

S. Silver, "Physical aspects of microwave radiation," *Proc. Third Tri-Service Conf. Biol. Effects of Microwave Radiating Equipments*, Berkeley, Calif., August 25–27, 1959; pp. 22–32.

S. Prausnitz and C. Süsskind, "Temperature regulation in laboratory animals irradiated with 3-cm microwaves," *Ibid.*, pp. 33–45.

C. Süsskind and P. O. Vogelhut, "Analytical and experimental investigation of unicellular organisms with 3-cm microwaves," *Ibid.*, pp. 46–53.

C. Süsskind and P. O. Vogelhut, "The use of electromagnetic radiation in elucidating biological phenomena," *Institute of Radio Engineers Mid-America Electronics Conference*, Kansas City, Mo., November 3–5, 1959.

C. Süsskind and P. O. Vogelhut, "Analytical and experimental investigation of unicellular organisms under microwave irradiation," *IRE-AIEE-ISA 12th Annual Conf. Electrical Techniques in Med. and Biol.*, Philadelphia, Nov. 10–12, 1959.

P. O. Vogelhut, "Microwaves as a tool in biological research," *3rd International Conf. Med. Electronics*, London, July 21–27, 1960.

S. Prausnitz, C. Süsskind, and P. O. Vogelhut, "Longevity and cellular studies with microwaves," *Proc. Fourth Tri-Service Conf. Biol. Effects of Microwave Radiation*, New York, August 16–18, 1960 (Plenum Press, New York); pp. 135–142.

T. Jaski and C. Süsskind, "Electromagnetic radiation as a tool in the life sciences," *Science* 133: 443–447, 1961.

S. Prausnitz and C. Süsskind, "Effects of chronic microwave irradiation on mice," *Proc. 4th International Conf. Med. Electronics*, New York, July 16–21, 1961.

C. Süsskind and P. O. Vogelhut, "Cavity-perturbation measurement of the effects of microwave radiation on proteins," *Proc. Instn El. Engrs.* 109(B), Suppl. 23: 668–669 and 682–685, 1961.

S. Prausnitz and C. Süsskind, "Effects of chronic microwave irradiation on mice," *Trans. Inst. Radio Eng.* (Prof. Gp. on Bio-Medical Electronics) BME-9: 104–108, 1962.